

# Serum zinc levels and its association with vitamin A levels among tuberculosis patients

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## Abstract

**Introduction:** One-third of the total human population is infected with the *Mycobacterium tuberculosis*. This bacterium causes illness in up to 9 million people annually and is responsible for three deaths every minute world-wide. **Objective:** To determine the association of serum zinc level with vitamin A level in active pulmonary tuberculosis (TB) cases. **Materials and Methods:** It was a cross-sectional study of 208 active pulmonary TB patients aged 18-55 years. Blood samples were obtained from these patients to determine the serum zinc and serum retinol levels. **Results:** The mean age of the patients was 30.56 ( $\pm 11.38$ ) years ranging from 18 years to 55 years. More than half (54.3%) of the patients were males and 63% were married. Body mass index of the patients was 18.40  $\pm$  3.10. The serum zinc and vitamin A levels among the patients were 9.60 ( $\pm 0.86$ )  $\mu\text{mol/l}$  and 0.77 ( $\pm 0.22$ )  $\mu\text{mol/l}$  respectively. However, haemoglobin, white blood cell, erythrocyte sedimentation rate, and serum albumin were 10.02 ( $\pm 1.33$ ) g/dl, 10076.01 ( $\pm 1822.67$ ) cell/mm<sup>3</sup>, 14.50 ( $\pm 2.95$ ) mm/h and 3.40 ( $\pm 0.32$ ) g/dl respectively. There was a strong correlation between serum zinc and vitamin A levels ( $r = 0.86$ ,  $P < 0.01$ ). Vitamin A levels were not significantly different among the different age groups; however, this was significantly ( $P = 0.001$ ) higher in male (0.82  $\pm$  0.23, 95% confidence interval [CI] = 0.77-0.86) patients as compared to females (0.71  $\pm$  0.20, 95% CI = 0.67-0.75). **Conclusion:** Zinc deficiency may indirectly influence the metabolism of Vitamin A via reduction of the levels of circulating proteins.

**Key words:** Deficiency, tuberculosis, vitamin A, zinc

## INTRODUCTION

Tuberculosis (TB) is one of the top 10 causes of illness, death, and disability world-wide and is the leading cause of death from a curable infectious disease.<sup>[1,2]</sup> It is estimated that approximately one-third of the world's population is infected with the *Mycobacterium tuberculosis* (hereafter called latent TB), with 8.8 million new cases during 2005 alone.<sup>[3,4]</sup> The annual number of new active TB cases is expected to increase to 9-10 million in the year 2010.<sup>[4]</sup> Smear-positive

TB, the most infectious form of the disease accounts for about 46% of these new cases.<sup>[3,5]</sup> World-wide, about 10% of those with latent TB are expected to develop active TB disease.<sup>[3,4]</sup>

Nutritional status is one of the most important determinants of resistance to infection, and it is well-established that nutritional deficiency is associated with impaired immune functions.<sup>[6]</sup> Although malnutrition limits cell mediated immunity and increases susceptibility to infection, infection can lead to nutritional stress and weight loss, thereby weakening immune function, and nutritional status.<sup>[7]</sup>

Vitamin A plays a central role in the growth and function of T and B cells, antibody responses, and maintenance of mucosal epithelia.<sup>[8]</sup> Vitamin A has an immunoprotective role against human TB. Low blood levels of vitamin A are associated with accelerated disease progression

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and increased mortality in human immunodeficiency virus-infected adults.<sup>[9]</sup> Vitamin A deficiency increases bacterial adherence to respiratory epithelial cells.<sup>[10]</sup> It has been known that vitamin A is excreted in the urine in patients with fever,<sup>[11]</sup> and this has been confirmed in subjects with acute infections, including pneumonia.<sup>[12]</sup>

Zinc is important for enzymes of all six classes as well as transcription and replication factors.<sup>[13]</sup> It is necessary for the normal function of the immune system.<sup>[14]</sup> Zinc has been shown to be essential in vitamin A metabolism because it is required to mobilize vitamin A from the liver.<sup>[15]</sup> Zinc deficiency may contribute to vitamin A deficiency even in the presence of adequate hepatic reserves of the vitamin. Animal studies have shown that zinc deficiency can interfere with intestinal retinol absorption.<sup>[16]</sup> Zinc deficiency also affects host defence in a variety of ways and it results in decreased phagocytosis and leads to a reduced number of circulating T cells and reduced tuberculin (purified protein derivative) reactivity, at least in animals.<sup>[17]</sup>

The objective of the present study was to determine the association of the serum zinc level with vitamin A level in active pulmonary TB cases.

## MATERIALS AND METHODS

### Study design and subjects

This was a cross-sectional study in which cases were out-patients with active pulmonary TB patients (new sputum smear positive) attended the Directly Observed Treatment Short-course center in the Department of Pulmonary Medicine, King George's Medical University, Uttar Pradesh, Lucknow. Selection of cases was based on the following criteria: Age 18-55 years; at least two sputum specimens positive for acid-fast bacilli by microscopy; and the clinical and the radiographic abnormalities consistent with pulmonary TB. Exclusion criteria for cases were as follows: previous anti-TB treatment, pregnancy, lactation, use of corticosteroids or supplements containing vitamin A or zinc during the previous month, moderate to severe injury or surgery during the last month, and diseases such as abnormal liver function as measured by elevated serum levels of aspartate amino transferase and alanine amino transferase, diabetes mellitus as measured by elevated fasting blood glucose levels, neoplasm as determined by the clinical examination, chronic renal failure as determined by elevated serum levels of urea and creatinine, and the congestive heart failure.

### Collection of blood samples

Blood samples (5 ml) were collected from enrolled patients via venipuncture. 2 ml blood were kept in Ethylene Diamine Tetra Acetic acid vial to determine hemoglobin (Hb), white blood cell (WBC) count, erythrocyte sedimentation

rate (ESR), and remain in the venipuncture. In the laboratory, serum was separated and analyzed for albumin, retinol, and zinc concentration.

### Biochemical analysis

Hb concentration and WBCs were measured directly using an automatic analyzer (Sysmex Microdilutor F-800, Kobe, Japan). ESR was determined directly using the Westergreen technique.<sup>[18]</sup> Serum Albumin was determined by the bromocresol green method.<sup>[19]</sup> Serum retinol was measured using the Retinol Binding Protein (human) enzyme-linked immunosorbent assay kit (Cat. No. AG-45A-0011EK-KI01) and zinc concentration was measured using the simple colorimetric method.<sup>[20]</sup>

### Ethical considerations

The study was approved by the Institutional Ethics Committee of King George's Medical University, Uttar Pradesh, Lucknow, India. Informed consent was obtained from each subject before the start of the study.

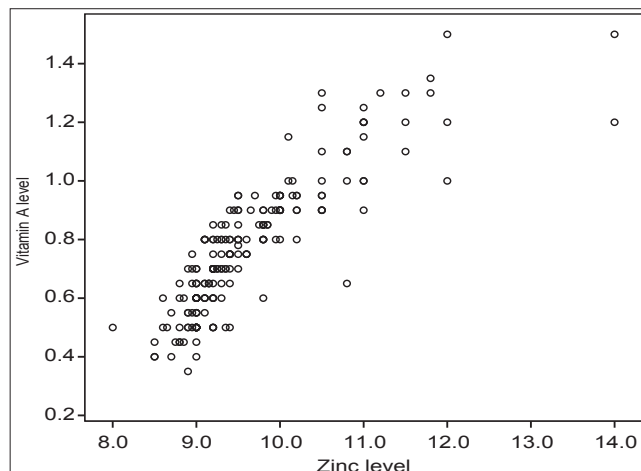
### Statistical analysis

The data collected were entered in Microsoft Excel sheet and checked for any inconsistency. The results are presented in mean ( $\pm$ SD) and percentages. The unpaired *t*-test is used to compare the differences in vitamin A levels between male/female and married/unmarried patients. The one-way analysis of variance is used to compare the vitamin A levels among different age groups. The 95% confidence interval (CI) of means is also calculated and presented. The Pearson correlation analysis is carried out to find out the correlation between vitamin A levels and serum zinc, Hb, serum albumin, WBC, and ESR. The multivariate linear regression is being carried out to assess the effect of serum zinc level to adjust confounding factors such as age, sex, and body mass index (BMI) of the patients on vitamin A levels. The  $P < 0.05$  is being considered as significant. All the analysis is being carried out by using the SPSS 15.0 version.

## RESULTS

A total of 208 patients of TB were studied to determine the serum zinc levels and its association with Vitamin A levels. The background characteristics of the patients are given in Table 1. The mean age of the patients was 30.56 ( $\pm$ 11.38) years with range 18-55 years. More than half (54.3%) of the patients were males and 63% of the patients were married. BMI of the patients was 18.40  $\pm$  3.10. The serum zinc and vitamin A levels among the patients were 9.60 ( $\pm$ 0.86)  $\mu$ mol/l and 0.77 ( $\pm$ 0.22)  $\mu$ mol/l respectively. However, Hb, WBC, ESR, and serum albumin were 10.02 ( $\pm$ 1.33) g/dl, 10076.01 ( $\pm$ 1822.67) cell/mm<sup>3</sup>, 14.50 ( $\pm$ 2.95) mm/h and 3.40 ( $\pm$ 0.32) g/dl respectively.

There was a strong correlation between serum zinc and vitamin A levels ( $r = 0.86$ ,  $P < 0.01$ ) [Figure 1]. Hb ( $r = 0.61$ ,  $P < 0.01$ ) and serum albumin levels ( $r = 0.87$ ,  $P < 0.01$ ) were also strongly correlated with the vitamin A levels; however, WBC ( $r = -0.60$ ,  $P < 0.01$ ) and ESR



**Figure 1:** Scatter plot of vitamin A ( $\mu\text{mol/l}$ ) and zinc ( $\mu\text{mol/l}$ ) levels (a strong correlation was found between serum zinc and serum vitamin A level ( $r = 0.86$ ,  $P < 0.01$ ))

**Table 1: Background characteristics of the patients**

| General characteristics             | No. (n = 208)            | %    |
|-------------------------------------|--------------------------|------|
| Age in years (mean $\pm$ SD), range | 30.56 $\pm$ 11.38, 18-55 |      |
| Sex                                 |                          |      |
| Male                                | 113                      | 54.3 |
| Female                              | 95                       | 45.7 |
| Marital status                      |                          |      |
| Married                             | 131                      | 63.0 |
| Unmarried                           | 77                       | 37.0 |
| Body mass index                     | 18.40 $\pm$ 3.10         |      |
| Vitamin A ( $\mu\text{mol/l}$ )     | 0.77 $\pm$ 0.22          |      |
| Zinc ( $\mu\text{mol/l}$ )          | 9.60 $\pm$ 0.86          |      |
| Hb (g/dl)                           | 10.02 $\pm$ 1.33         |      |
| WBC (cells/mm <sup>3</sup> )        | 10076.01 $\pm$ 1822.67   |      |
| ESR (mm/h)                          | 14.50 $\pm$ 2.95         |      |
| Serum albumin (g/dl)                | 3.40 $\pm$ 0.32          |      |

General characteristics and mean of the biochemical variables of the pulmonary tuberculosis patients, Hb: Hemoglobin, WBC: White blood cell, ESR: Erythrocyte sedimentation rate

**Table 2: Correlation between vitamin A level with zinc and biochemical parameters**

| Parameters                   | Vitamin A |
|------------------------------|-----------|
| Zinc ( $\mu\text{mol/l}$ )   | 0.86*     |
| Hb (g/dl)                    | 0.61*     |
| WBC (cells/mm <sup>3</sup> ) | -0.60*    |
| ESR (mm/h)                   | -0.79*    |
| Serum albumin (g/dl)         | 0.87*     |

\*Pearson correlation coefficient,  $P < 0.01$ ; That serum zinc, Hb and serum albumin were strongly correlated with vitamin A level. However, WBC and ESR were negatively correlated, Hb: Hemoglobin, WBC: White blood cell, ESR: Erythrocyte sedimentation rate

( $r = -0.79$ ,  $P < 0.01$ ) were negatively correlated with the vitamin A levels [Table 2].

Table 3 shows the effect of background characteristics of patients on vitamin A levels. The vitamin A levels was insignificantly ( $P > 0.05$ ) higher in the age group of 31-40 years of patients ( $0.80 \pm 0.27 \mu\text{mol/l}$ , 95% CI = 0.71-0.90) as compared to below 20 years ( $0.79 \pm 0.23$ , 95% CI = 0.69-0.90), above 50 years ( $0.78 \pm 0.27$ , 95% CI = 0.65-0.91), 20-30 years ( $0.76 \pm 0.20$ , 95% CI = 0.72-0.79) and 41-50 years ( $0.74 \pm 0.19$ , 95% CI = 0.66-0.82). The vitamin A levels were significantly ( $P = 0.001$ ) higher in male ( $0.82 \pm 0.23$ , 95% CI = 0.77-0.86) patients as compared to females ( $0.71 \pm 0.20$ , 95% CI = 0.67-0.75). There were no significant differences ( $P > 0.05$ ) in the levels of vitamin A between married and unmarried patients.

Table 4 presents the results of multivariate linear regression analysis determining the effect of serum zinc levels on vitamin A adjusted for age, sex, and BMI of the patients. The adjusted regression coefficient for the serum zinc level remained same as in unadjusted analysis, which was 1.35 ( $P < 0.0001$ ) indicating that there was no confounding effect of age, sex, and BMI of the patients in the level of vitamin A.

**Table 3: Vitamin A levels by background characteristics of the patients**

| Background characteristics | No. of patients | Vitamin A level (mean $\pm$ SD), 95% CI | 95% CI of mean |
|----------------------------|-----------------|---|----------------|
| Age (years)                |                 |   |                |
| <20                        | 21              | 0.79 $\pm$ 0.23                         | 0.69-0.90      |
| 20-30                      | 105             | 0.76 $\pm$ 0.20                         | 0.72-0.79      |
| 31-40                      | 36              | 0.80 $\pm$ 0.27                         | 0.71-0.90      |
| 41-50                      | 27              | 0.74 $\pm$ 0.19                         | 0.66-0.82      |
| >50                        | 19              | 0.78 $\pm$ 0.27                         | 0.65-0.91      |
| Sex*                       |                 |   |                |
| Male                       | 113             | 0.82 $\pm$ 0.23                         | 0.77-0.86      |
| Female                     | 95              | 0.71 $\pm$ 0.20                         | 0.67-0.75      |
| Marital status             |                 |   |                |
| Married                    | 131             | 0.76 $\pm$ 0.23                         | 0.72-0.80      |
| Unmarried                  | 77              | 0.78 $\pm$ 0.22                         | 0.73-0.83      |

\* $P < 0.001$  (significant); The level of serum vitamin A in different age groups, sex and marital status, CI: Confidence interval

**Table 4: Effect of serum zinc levels on vitamin A levels adjusted for age, sex, and BMI of the patients**

| Parameters                 | $\beta$ -coefficient | 95% CI of $\beta$ | P value  |
|----------------------------|----------------------|-------------------|----------|
| Zinc ( $\mu\text{mol/l}$ ) | 1.35                 | 1.14-1.5          | <0.0001* |
| Age (years)                | -0.02                | -0.002-0.001      | 0.65     |
| Sex                        | 0.03                 | -0.008-0.06       | 0.13     |
| BMI                        | 0.001                | -0.008-0.06       | 0.13     |
| Constant                   |                      | -1.30             |          |

$R^2 = 0.74$ ; That there was no confounding effect of age sex and BMI in the level of serum vitamin A ( $P < 0.0001$ ), BMI: Body mass index

## DISCUSSION

Zinc deficiency affects the host defenses in a variety of ways and it results in decreased phagocytosis and leads to a reduced number of circulating T-cells and reduced tuberculin reactivity, at least in animals.<sup>[21]</sup> *In vitro* cellular killing by macrophages was found to be reduced during zinc deficiency and rapidly restored after zinc supplementation.<sup>[22]</sup> Zinc has essential role in vitamin A metabolism. Zinc supplementation has a beneficial effect on vitamin A metabolism, which has an important role in TB. An adequate supply of zinc may also limit free radical membrane damage during inflammation.<sup>[23]</sup>

In the present study, a total of 208 TB patients were assessed for their status of zinc level and its association with vitamin A level. The serum zinc and vitamin A levels among the patients were  $9.60 (\pm 0.86) \mu\text{mol/l}$  and  $0.77 (\pm 0.22) \mu\text{mol/l}$  respectively. However, Hb, WBC, ESR and serum albumin were  $10.02 (\pm 1.33) \text{ g/dl}$ ,  $10076.01 (\pm 1822.67) \text{ cell/mm}^3$ ,  $14.50 (\pm 2.95) \text{ mm/h}$  and  $3.40 (\pm 0.32) \text{ g/dl}$  respectively. A study from Rwanda reported vitamin A deficiency among adults with TB. Concentration of vitamin A was found lower in TB patients than controls;<sup>[24]</sup> however, in an Indian study, the low vitamin A levels observed in TB patients returned to normal at the end of anti-tuberculosis treatment without vitamin A supplementation.<sup>[25]</sup>

The lack of a reliable biological marker for zinc deficiency may mask the real role of this trace element in the genesis of vitamin A deficiency in the studied subjects, whereas, it occurs in other micronutrients, due to a strict homeostatic control, serum zinc levels can be found within normal limits even in individuals with the low body stores of this element; however, despite these limitations, serum zinc levels are still the most widely used biomarker for zinc status of the organism since other biomarkers also showed limitations in addition to present major technical difficulties in its execution.<sup>[26]</sup> In our study, there was strong correlation between serum zinc and vitamin A levels ( $r = 0.86$ ,  $P < 0.01$ ). Coutsooudis *et al.* have also reported a positive correlation between vitamin A and serum zinc levels.<sup>[27]</sup> In our study, Hb ( $r = 0.61$ ,  $P < 0.01$ ) and serum albumin levels ( $r = 0.87$ ,  $P < 0.01$ ) were strongly correlated with the vitamin A levels. However, WBC ( $r = -0.60$ ,  $P < 0.01$ ) and ESR ( $r = -0.79$ ,  $P < 0.01$ ) were negatively correlated with the vitamin A levels. To best of our knowledge, none of the study was found to correlate these micronutrients with vitamin A; hence comparison with existing literature could not be made.

In our study, the vitamin A levels were not significantly different among the different age groups. However, this was significantly ( $P = 0.001$ ) higher in male ( $0.82 \pm 0.23$ , 95%

CI = 0.77-0.86) patients as compared to female ( $0.71 \pm 0.20$ , 95% CI = 0.67-0.75). A study on pre-school children reported no significant difference in vitamin A between male and female.<sup>[28]</sup> This difference might be due to that they studied on pre-school children while our study was on adult pulmonary TB patients. There were no significant differences ( $P > 0.05$ ) in the levels of vitamin A between marital statuses of the patients. The multivariate linear regression analysis determining the effect of serum zinc levels on vitamin A adjusted for age, sex, and BMI of the patients indicated that the adjusted regression coefficient for serum zinc level remained same as in unadjusted analysis, which was 1.35, indicating that there was no confounding effect of age, sex, and BMI of the patients in the level of vitamin A. The results of this study also raise the possibility that zinc deficiency may indirectly influence the metabolism of other nutrients in men via reduction of the levels of circulating proteins. One example, already extensively investigated in animals and to a lesser degree in men, is the role of zinc deficiency on vitamin A metabolism, mainly through its effect on retinol binding protein.<sup>[15]</sup> A variety of other nutrients, which rely on transport proteins, such as iron and transferrin, may have their metabolism altered by severe zinc deficiency with decreased nutrients being transported for organ utilization.

## CONCLUSION

Thus on the basis of the above findings it could be concluded that nutritional status of the patients is an important determinant to resistance against infections. The zinc deficiency may indirectly influence the metabolism of Vitamin A via reduction of the levels of circulating proteins. Hence, it is advisable that zinc supplementation along with vitamin A may be helpful in attaining disease condition.

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